EXHIBIT A

Raman Spectral Intensities of Nucleic Acid at 251 nm-Excitation from E. coli and E. coli-Antibody Complexes

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Abstract

Escherichia coli bacteria in the logarithmic growth phase have been investigated by UV resonance Raman spectroscopy. Bacterial Raman spectra excited at 251 nm reflect nearly exclusively the nucleic acid composition even though a large fraction of the bacterial mass is composed of protein. The Raman spectral cross sections have been measured and compared to calculated values. It has been demonstrated that if bacteria are grown under controlled (logarithmic growth) conditions which give rise to organisms of known biochemical composition, the Raman spectral intensities of *E.coli* can be predicted quantitatively from the knowledge of component nucleic acid base cross sections.

The great selectivity of the nucleic acid excitation in the presence of a large excess of antibody is demonstrated in this work as well. UV resonance Raman spectra of bacteria in the presence of up to one million-fold excess of antibody in aqueous suspension have been excited at 251 nm. Such spectra contain only nucleic acid peaks due to bacteria.

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INTRODUCTION

Conventional Raman spectroscopy is well suited for studying biological molecules because of its high spectral information content, and because water does not significantly interfere. Resonance Raman spectroscopy like conventional Raman is easily applied to aqueous solutions, but offers different information. The higher sensitivity and selectivity provided by resonance enhancement may more than offset the loss of spectral information. The development of UV laser excitation sources has permitted the extension of resonance Raman spectroscopy to the study of molecules with electronic transitions in the near-, mid-, and far-UV regions [1-2]. This already has been used to advance our fundamental understanding of molecular structure and dynamics. UV Resonance Raman (UVRR) spectroscopy has been a success as a probe to study purine and pyrimidine structure,[3,4] polynucleotides [5], and nucleic acids [6,7], as well as aromatic amino acids in proteins, [8-10] peptide bonds [11,12] and dynamic structural changes in proteins.[13,14] For oligo- and poly-nucleotides, the Raman hypochromism observed under the resonance conditions is very sensitive to base-base stacking interactions.[15] Raman spectra of aromatic amino acids also have been obtained at 240 [16,17], 248 [18], 253 [19] and 257 [8] nm excitation. Most recently, structure changes [20,21] and the excited-state relaxation rate[22] of hemoglobin have been studied by UV resonance Raman.

Resonance Raman spectroscopy is a powerful technique for studying DNA conformational changes[15], and it gives very promising results in the selective investigation of chromophores in molecular complexes such as nucleic acid bases within DNA[5]. The purine and pyrimidine bases of the nucleic acids absorb strongly at wavelengths shorter than 280nm[23]. Vibrational coupling between deoxyribosyl and thymine moieties and structural implications have been studied [24] with 244nm excitation. The UV resonance Raman spectra of nucleic acids and bases often have been obtained at 257 nm. This wavelength has been used in part because it is close to the maximum of the UV absorption for nucleic acids. However, for many years this was the only strong CW exciting line available in this region. At excitation wavelengths shorter than 257nm resonance Raman spectra can be obtained within the finger-print region without the fluorescent interference of aromatic amino acids always present in proteins.

UV resonance Raman has been used increasingly to study both biomolecules and cells[25-27]. Interactions of DNA with basic peptides[28], DNA-β-propiolactone interaction [29], and the vibrational modes of uracil [30,31] and guanine derivatives [32] were investigated with 257nm excitation at an early stage. The spectra of nucleic acids of living cells were first observed [33] by UVRR at 257nm excitation. Later it was demonstrated [34] that many of the spectral features of the single-cell nuclear DNA essentially arise from cellular purine derivatives. UV resonance Raman spectroscopy has been utilized[35] to observe directly structural features of the recently described nucleic acid single-stranded helix $d(A^{+}\text{-}G)_{10}$ with 250 nm excitation. The first UV resonance Raman studies of whole bacterial cells were accomplished with 242nm excitation.[36] The effects of cultural conditions of bacteria were evident with 242 nm excited spectra[37] but not with 222 or 231 nm excitation. *E.coli* spectra excited with 251nm light resembled spectra excited at 242nm, but showed [38,39] weaker contributions from protein. For example, nearly all tyrosine and tryptophan contributions are absent in the 251 nm excited spectra. Medical applications show considerable promise. For example, spectra of DNA in colon mucosa [40] excited at 251nm have been used for the purpose of rapid cancer screening.

For many Raman studies it is helpful to determine the Raman spectral cross section, since the resulting intensity information can be used to determine the concentrations of species of interest. Nucleic acid base and mononucleotide UVRR intensity profiles give information on the excited state geometries of the molecules. There have been several studies of resonance Raman cross section excitation profiles [41-46]. Few data have been obtained with precisely 251nm excitation. However, Raman cross sections have been measured [47] for the major peaks from nucleotides and polynucleotides with 252nm excitation. Frequency and intensity changes associated with base pairing and stacking have been analyzed as well. Low-resolution excitation profiles have been constructed [48] for the strongest bands for the four ribonucleotides AMP, GMP, UMP and CMP with laser excitation from 299 to 200 nm including 253nm. Absolute cross sections have been obtained [49] for guanine bound to platinum in mono- and di-nucleotides with 252nm excitation. Raman cross-sections of the single-stranded DNA genome of filamentous virus *fd* have been measured [50] at 229, 238, 244 and 257nm excitation. Assignments and cross section of DNA and protein constituents of viruses were

obtained[51] by UV resonance Raman with excitation of 257, 244, 238 and 229nm as well. Raman cross-sections for some individual aromatic amino acids have been obtained [52] with excitation at 245nm. For a variety wavelengths [46-48,51,55] in the UV, information regarding the selectively of excitation of aromatic amino acid residues in proteins or purine and pyrimidine bases in nucleic acids is available.

Quantitative Raman intensity data from bacteria are needed if the sensitivity of UVRR spectroscopy is to be determined in bacterial detection. In this work, the bacterium *E. coli* has been investigated by UV resonance Raman spectroscopy with the excitation wavelength of 251nm. Specifically, an attempt is made to determine if nucleic acid bases can be excited and determined quantitatively from whole bacterial cells. The use of 251nm light for excitation is ideal for the purpose since protein excitation is minimal at 251nm. *E.coli* is a desirable bacterium for study because its cellular composition is well known for cultures grown under defined conditions. Normally, if reproducibility of composition is desired, organisms in the logarithmic growth stage are used. Typically, the chemical composition of log phase *E. coli* dry mass shows [53]55.0% protein, 20.5% RNA, 3.1% DNA, and 20.6% of other constituents including lipid, lipopolysaccharide, murein and glycogen, which form the total of 96.1% of macromolecules. The rest 3.9% are some inorganic ions and various building blocks, metabolites and vitamins. The average total weight of one cell of *E.coli* in the log phase is 9.5 $x10^{-13}$ g according to Neidhardt [53], but the dry weight is $2.8x10^{-13}$ g. The cell contains much water.

Most significantly the numbers and kinds of nucleic acid base pairs in DNA and RNA are known for log-phase *E.coli*. The Raman spectral intensities for bacteria can be calculated as the sum of spectral intensities of the known numbers and kinds of bases within the nucleic acids. Some uncertainty will be associated with effects such as hypochromism and internal absorption. This work will attempt to determine if the UVRR spectral intensities of bacterial *E.coli* can be predicted quantitatively from the knowledge of nucleic acid free base cross sections given the numbers and kinds of base pairs. If bacteria are grown under controlled conditions which give rise to organisms of known biochemical composition, this may be possible. Raman spectra of *E.coli* excited at 251nm

have been obtained. The spectral cross sections have been measured and compared to the calculated values in this study.

E.coli-antibody complexes have been studied as well. Such studies are expected to show the extent to which resonance Raman spectra of nucleic acids in bacteria-antibody complexes can be excited selectively. Little is known about the resonance Raman spectra of antibodies. Since antibody contains aromatic amino acids, it is expected that these will be UV excited to a limited extent. To the extent that the aromatic amino acids of the antibody are excited, and these peaks overlap with nucleic acid peaks, they can be expected to interfere with nucleic acid spectra. The degree to which this will occur is unknown.

EXPERIMENTAL SECTION

Instrumentation A quasi-continuous third/fourth harmonic tunable, mode-locked coherent Ti:sapphire (Ti:Sa) Ar^+ laser system was used as the excitation source, which produces 203ps pulses at a 76 MHz repetition rate [54] and is continuously tunable over the 200-300 nm range. Raman spectra were taken with the excitation at 251 nm with a typical output power of around 25mW. A flow system was used to circulate the sample at a flow rate of about 15mL/min by means of a Masterflex pump in a closed loop consisting of the pump and a flat quartz sample cell with a path length of about 5-8 mm. The laser beam impinged on the flat quartz cell surface at an angle of about 30° to the optic axis, defined by the spectrometer, to avoid collecting reflected laser light. The power of the UV light hitting the sample cell surface varied between 8 and 15 mw. The Raman-scattered light was collected along the direction perpendicular to the sample surface. A one-meter-long f/8 spectrometer (SPEX 1000M) was used to disperse the light at 0.4 nm/mm with a single 2400 grooves/mm grating across a liquid-nitrogen-cooled CCD detector (1100PB, Princeton Instrument, Inc.). A solid edge filter (Barr Associates Inc., Westford, MA) was used to rid spectra of most Rayleigh scattering. At 251 nm, the incident laser beam was estimated to give a spot area of 10x20 μm^2 to 50x50 μm^2 at the sample cell. The Raman shift axis of the CCD spectra was calibrated by ethanol. All the raw data were collected digitally and imported into GRAMS 386 software (Galactic Industries Co., Salem, NH) for processing and display.

Chemicals Nucleosides (A, G, U, C, T), aromatic amino acids (Tryp, Tyr), Na_2SO_4 and D_2O (99.9%) were purchased from Sigma Chemical (St. Louis, MO). Solutions of nucleosides and aromatic amino acids were prepared in the concentration range of 0.2-2.0 mM in H_2O.

Microbiological Samples *Escherichia coli* was obtained from the Microbiology Dept., University of Rhode Island, Kingston, RI. *E.coli* antibodies (O&K) B65001R, IgG-Rabbit were purchased from BioDesign Intl., Kennebank, ME. Stock cultures were maintained on Trypticase soy agar slants at 4°C, following incubation at 37°C for 24 h. A loopful of bacteria was resuspended in 5 ml nutrient broth and 50 ml Trypticase soy broth (TSB) without glucose (1.5% Trypticase, 0.5% Soytone and 0.5% NaCl), and incubated with shaking (250rpm) for 15-18 h at 37°C. The bacteria were washed with 4-5 ml of sterile 0.85% saline, then twice centrifuged to remove any culture medium from the bacterial pellet. The bacteria were resuspended in 5 ml of 0.025 M phosphate buffer (PH=6.8) before use. To prepare the bacteria antibody mixtures, antibodies were add to the above suspension to achieve antibody concentrations of 0.05, 0.5 5.0 and 50 µl/ml, respectively.

The bacterial samples were enumerated by three methods. The cell concentration was first estimated by reading the optical density (OD) at 600 nm wavelength with a Baush&Lomb Spectrophotometer. A total direct count of a serial dilution was then determined using a standard Hemacytometer (Reichert, Baffalo, NY). The number of cells in three squares were counted and then averaged. The concentration was then calculated using the following formula:

Concentration = Average number of cells/square x 16 x10^4 x dilution factor

The third method used was a standard plate count. The number of viable cells was determined by counting the colony forming units. Serial dilutions were performed to provide between 25 and 250 colony-forming units per ml plated. Aliquots were plated and then incubated for 24 hours at 37 °C. The concentration of viable cells/ml was then calculated using the following formula:

Viable cells = Average number of cells x dilution factor

The average of the direct count and colony forming unit count was used in the calculation of the bacterial concentrations used in cross-section determination.

Methods of Calculation

Raman Scattering Cross Section The intensity measurements were obtained by means of comparison with the Raman intensities of an internal standard. Use of an internal standard eliminates the need to determine notoriously difficult absolute intensity measurements. In this work aqueous Na_2SO_4 is used as the internal standard in all calculations of Raman spectral cross-sections.

The Raman cross section (σ_N) of a Raman band of a sample at frequency ν_N is determined by comparison of its peak height (I_N) with the peak height (I_S) of the internal standard of known absolute Raman scattering cross section (σ_s) at frequency ν_S by using the equation: [55]

$$\sigma_N = \sigma_s (I_N/I_S) (C_S/C_N)\{(\nu_0 - \nu_S)/(\nu_0 - \nu_N)\}^4 \quad (1)$$

where ν_0 is the laser frequency. C_N and C_S are the molar concentrations of the sample and standard, respectively.

The prominent UV Raman band of the internal standard sodium sulfate at 981 cm^{-1} is assigned to the symmetrical stretching vibration of the SO_4^{2-} ion. The absolute Raman cross section of this band can be determined [55,56] as a function of the UV excitation wavelength by the equation :

$$\sigma_s = K\nu_0 (\nu_0 - \nu_s)^3 \{(\nu_e^2 + \nu_0^2)/(\nu_e^2 - \nu_0^2)^2 + K'\}^2 \quad (2)$$

where ν_e is the frequency of the resonant electronic transition. The best-fit wavelength, λ_e, for the resonant transition is 146 nm for sulfate. ν_0 is the laser frequency, and ν_s is the frequency of the Raman transition of sulfate which corresponds to 981 cm^{-1}. K is a factor containing the Franck-Condon overlap in the resonant excited state and K' is a phenomenological parameter representing the off-resonant contributions of higher lying transitions, which flatten the curves at longer wavelengths. In this work, $K=0.89\times10^{-28}$ cm^2/molecule•sr, $K'=3.4\times10^{-10}$ cm^2 are used[55].

Calculated Raman Spectral Cross Sections of Nucleic Acid Peaks of Bacteria

The Raman cross-sections of the nucleic acid peaks belonging to the bacterial cell have been calculated by summation of the cross sections of the known numbers and kinds of bases composing the nucleic acid. The number and composition of bases from DNA and RNA for *E.coli* B/r protoplasm is known[53] and listed in Table 1.

Table 1. Composition of E.coli B/r (μmol/g of dried cells)

	RNA nucleotides	DNA nucleotides	Protein amino acid
AMP	165	24.6	-
GMP	203	25.4	-
CMP	126	25.4	-
UMP	136	-	-
TMP	-	24.6	-
Trp	-	-	54
Tyr	-	-	131

With the information in Table 1 and the knowledge of the average weight of one dry cell of *E.coli* (2.8×10^{-13} g/cell), the number of bases, A, G, C, U, T, and the number of aromatic amino acid units of Tryptophan(Tryp) and Tyrosine(Tyr) in one *E.coli* cell have been calculated and listed in Table 2.

Table 2. Number of Molecules of A, G, C, U, T, Trp and Tyr Units in One Cell of *E. coli*

Component	Molecules $\times 10^6$/cell
A	32.0
G	38.6
C	25.6
U	22.9
T	4.15
Trp	9.11
Tyr	22.1

The Raman spectral cross section of nucleic acid peaks belonging to a single bacterial cell (σ_{cal}) can be calculated from the summation of the absolute cross-sections of the Raman band components (σ_C):

$$\sigma_{cal} = \sum f_C \sigma_C \qquad (3)$$

where f_C is the number of each component in one cell of *E.coli*. The Raman cross-sections belonging to selected modes of adenosine, guanosine, cytidine, uridine, thymidine and the 1610-1620 cm^{-1} bands of tryptophan and tyrosine have been measured with 251nm excitation in this work since these are not available in the literature.

The reported Raman cross-sections of nucleosides are averages of multiple independent experiments exhibiting band intensity deviations of less than ±10%. The scattering background due to the quartz cell and solvent was subtracted from all bacterial spectra.

RESULTS SECTION

Aqueous nucleoside solutions have been excited at 251nm. The Raman cross-sections of modes of adenosine, guanosine, cytidine, uridine and thymidine have been determined and are compared with 252 and 257 nm excitation results determined elsewhere listed in Table 3.

Table 3. Cross Sections of Prominent Raman Bands of Nucleosides in UVRR Spectra Excited at 251, 252 [47] and 257 [51] nm

	Cross Section (millibarns)		
Raman Band (cm^{-1})	251 nm	252 nm	257 nm
Adenosine			
1336	382	401	367
1482	231	265	211
1506	95	-	109
1580	85	114	76
Guanosine			
1332	163	-	80
1361	73	63	36
1485	415	391	208
1575	197	195	114
Cytidine			
1252	17	-	24
1293	15	20	20
1527	49	47	29
1650	39	29	18
Uridine			
1230	136	135	-
1394	32	39	-
1476	17	22	-
1625	62	51	-
Thymidine			
1190	27	-	24
1244	61	-	58
1374	80	-	85
1414	13	-	15
1652	102	-	88

Bacterial Raman spectra excited at 257nm and 251nm are similar in appearance[25]. However, 251nm excitation provides excellent selectivity in nucleic acid detection and better quality spectra. With 257nm-excitation aromatic amino acid fluorescence begins to interfere[25] significantly beyond 1000cm^{-1}. In contrast, with 251nm-excitation fluorescence interference within the finger-print region is negligibly small.

Raman spectra excited at 251nm for a series of diluted *E.coli* log phase suspensions in H_2O and D_2O with sodium sulfate as internal standard are shown in Figure 1 and Figure 2 with the background subtracted. The measured Raman cross-sections for the major peaks of a typical serial dilution experiment involving *E.coli* are listed in Table 4.

Table 4. Experimental Raman Cross-Sections of A Typical Serial Dilution of Log Phase *E.coli* Excited at 251nm (6.25 x10^8 cells/OD)

OD	conc.(M) x10^{-12}	Na_2SO_4 (M)	Cross-Sections (x 10^{-18}) 1240 cm^{-1}	1334 cm^{-1}	1485 cm^{-1}	1575 cm^{-1}	1618 cm^{-1}
0.98	1.01	0.075	-	8.36	17.4	7.59	3.20
1.96	2.03	0.15	2.66	7.67	19.3	7.01	3.67
3.92	4.07	0.30	2.78	7.96	18.4	7.54	2.94
7.84	8.14	0.40	3.49	7.91	18.3	7.53	2.70

In Table 5 the average values of the Raman cross-sections for each major bacterial mode are compared with values calculated from the sum of identical nucleic acid base contributions. No effects due to hypochromism or internal absorption are included in the calculations.

Table 5. Experimental and Calculated Cross-sections of Prominent Raman Bands for *E.coli* (Log Phase) Excited at 251nm (cm^2/mol•sr x10^{-18})

Raman bands(cm^{-1})	1240	1339	1485	1575	1615
Cross-sections(measured)	3.02	8.97	19.3	7.96	3.29
Peak components	T+U	A	A+G-	A+G	Trp+Tyr
Cross-sections(calculated)	3.48	12.1	23.6	10.3	1.1

The 1615 cm^{-1} Raman band of the bacteria is composed of tyrosine and tryptophan and a negligible component due to phenylalanine. The cross-sections of aqueous tyrosine and tryptophan modes have been measured in this work at 251 nm excitation. The values used to calculate the cross-section of this 1615 cm^{-1} peak are 33.5(tyr) and 38 (trp) millibarns, respectively.

Figure 3 shows the Raman spectra of *E.coli*-antibody complexes obtained from solutions with different amounts of antibody added. In all cases there is a great excess of antibody. Because the 1648cm^{-1} water band has not been subtracted, the spectra are limited to the prominent 1339cm^{-1} and 1485 cm^{-1} bands.

DISCUSSION

Raman Bacterial Cross-Sections

The Raman cross-sections have been calculated assuming that bacterial peaks excited by 251nm laser light are due to excitations of purine and pyrimidine base contributions from the nucleic acids. For the purposes of comparison with experimental results, the calculated cross-sections are based upon the assumption that hypochromism and internal absorption can be ignored. Basically, given the known average A, G, C, T, U content per *E. coli* cell, the cross-sections have been calculated as the sum of the individual base contributions.

Table 4 shows the measured cross-sections from one typical *E.coli* log phase serial dilution experiment. The standard deviation is only 0.77 ((18.4±0.7)x10^{-18} cm^2/mol•sr) for the 1485 cm^{-1} Raman band based on the peak height data for this typical

serial dilution experiment. But for all the multiple independent experiments, the standard deviation is much larger ((19.6±5.1)x10^{-18} cm^2/mol•sr) for the same band. Constituents of *E.coli* in the log phase A, G, C, T, U are well-defined, but in practice the enumeration of the organisms proved the greatest source of variability. Since only live organisms were counted in the colony forming unit count, but live and dead organism will give similar Raman spectra, our cross-sections may be too large to the extent that we "undercounted" the cells. It is believed that under the conditions applied that very few cells died. There is also the possibility of " undercounting" due to the clumping of bacteria. However, this problem is minimized for *E. coli* since these organisms do not tend to aggregate.

The comparison of the calculated with the experimental cross-sections in Table 5 shows that experimental values are close to the calculated ones. In addition, the experimental values are modestly lower in magnitude. This lower magnitude of the measured cross-sections compared to the calculated ones is attributed to hypochromism.

Most significantly, the experimental and calculated cross-sections are very similar in magnitude. This shows that to a good approximation the intensities of nucleic acid resonance Raman peaks of *E. coli* bacteria excited at 251 nm can be estimated from cellular nucleic acid base composition. This strongly suggests that the intensities of Raman spectra of other bacterial species can be predicted successfully as well, if the nucleic acid composition is known. Such calculations now allow the direct comparison of the intensities of resonance Raman detection with detection using other spectroscopic methods for which absolute cross-sections are known.

Bacteria-Antibody Complexes

The study of bacteria-antibody complexes is of great interest due to the widespread use of immunology-based bacterial detection and identification methods. In this study, we have attempted to determine the extent to which bacterial nucleic acid can be studied in the presence of excess antibody without the antibody spectra seriously interfering. Toward this end we have obtained spectra excited at both 242 and 251 nm. The antibody/bacteria ratio has been varied from 2000/1 to 1,000,000/1. With 242nm excitation[57], the nucleic acid spectra clearly are visible among the aromatic amino acid peaks at the ratio of 2000/1 but cannot be detected at the ratio of 10,000/1.

In contrast with 251nm excitation, Fig.3, no aromatic amino acid peaks can be observed even at the antibody/bacterium ratio of $10^6/1$. We have already shown the resonance Raman excitation at 257 nm even in the presence of fluorescence interference, allows the detection of only a handful of bacteria. This latest finding suggests the possibility of a new method for bacterial detection based upon the selective resonance Raman excitation of bacteria attached to immobilized antibody.

Specifically, the present data suggest that it will be possible to use standard methods of immunomagnetic isolation followed by resonance Raman detection of the selectively attached bacteria in a very rapid detection process which will not require special reagents other than immunomagnetic beads or other immobilized antibody. Micro-sized beads having a very large surface/volume ratio will allow the bacteria and antibody to be isolated in a small volume which can be excited by a focused laser beam of modest power. The presence of a huge excess of antibody should allow the detection of very small numbers of bacteria directly and rapidly. It is expected that the selectivity of excitation will be very high in the 248-257 nm region. Nucleic acid Raman cross-sections vary little in the range of 251-257 nm, but the reduction of fluorescence in the fingerprint region for 248-251 nm excitation points toward that wavelength range being optimal for selective detection of bacteria attached to antibody.

Conclusions

High quality resonance Raman spectra of *E.coli* have been excited by 251 nm light. Spectra show a high selectivity for nucleic acids and the absence of fluorescence in the finger-print region. High signal-to-noise ratios permit precise quantitative determination of the Raman peak height for all prominent Raman bands in bacteria due to nucleic acids. The comparison of the measured and calculated values of the cross-sections of *E.coli* (log phase) modes shows that experimental values to be about 25% less than calculated values. Raman cross-sections of nucleoside spectra excited at 251 nm are very close in value to those reported[47] at 252 nm. This agreement between our data of standard nucleosides excited at 251 nm with data excited at 252 nm demonstrate that our method of spectral measurement closely approximates the results determined for nucleosides independently in other laboratories.

It has been demonstrated that *E. coli* Raman spectra excited at 251 nm can be approximated as due to the simple sum of purine and pyramidine base Raman contributions. However, hypochromism appears to be in the range of 20-30%. Bacterial Raman spectra excited at 251 nm reflect nearly exclusively nucleic acid composition even though a large fraction of bacterial mass is composed of protein. The single protein peak at 1615 cm^{-1} exhibits strong hyperchromism.

The great selectivity of the nucleic acid excitation in the presence of a large excess of antibody protein is demonstrated in this work as well. This result may provide the basis for the rapid quantitation of bacteria using immunological methods. UV resonance spectra of bacteria in the presence of up to one million-fold excess of antibody have been excited at 251 nm. Such spectra contain only nucleic acid peaks due to bacteria. This result suggests that it may be possible to detect and enumerate bacteria attached to immobilized antibody. It has already been demonstrated that it is possible to detect as few as 10 bacteria using UVRR spectroscopy[58]. If practical means can be devised for specific application to immobilize bacteria selectively and quickly, UVRR spectroscopy promises to provide a rapid and nearly reagentless quantitative means of bacterial detection and identification. This requires as well that the bacteria and antibody can be collected in a focused laser beam.

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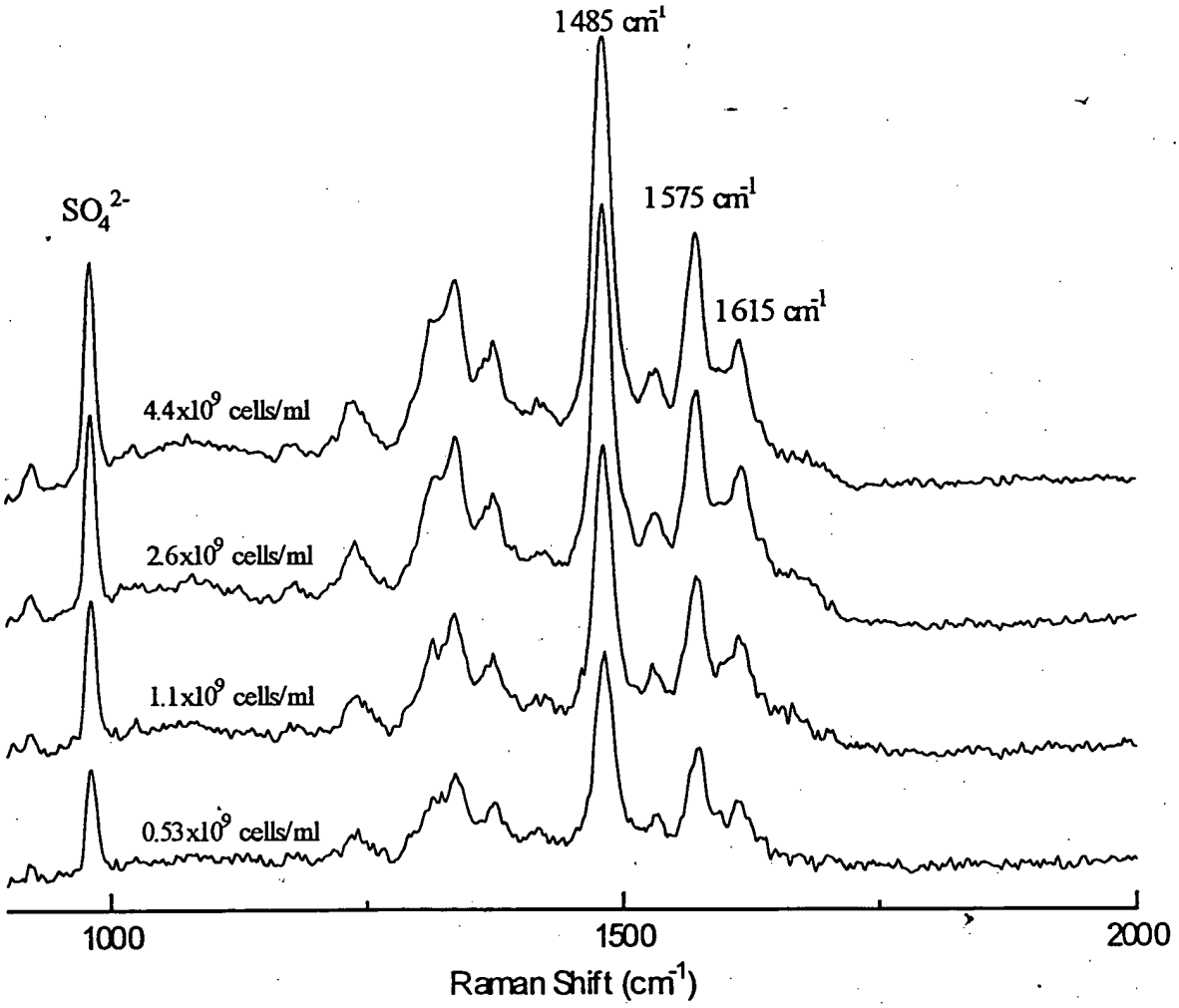

Figure 1. Raman Spectra of Serial Diluted *E.coli* Log-Phase Suspensions in H_2O

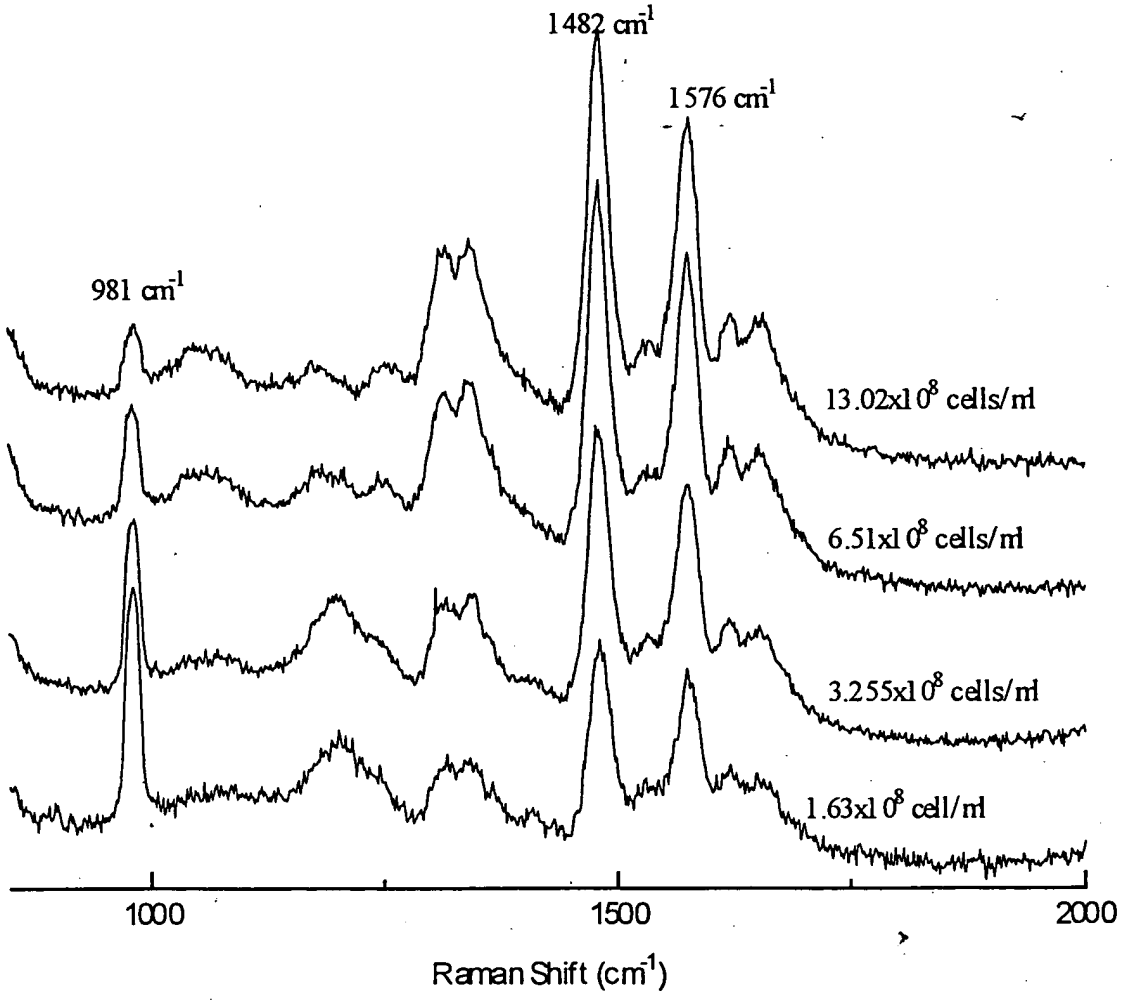

Figure 2. Raman Spectra of Serial Diluted *E.coli* Log-Phase Suspensions in D_2O

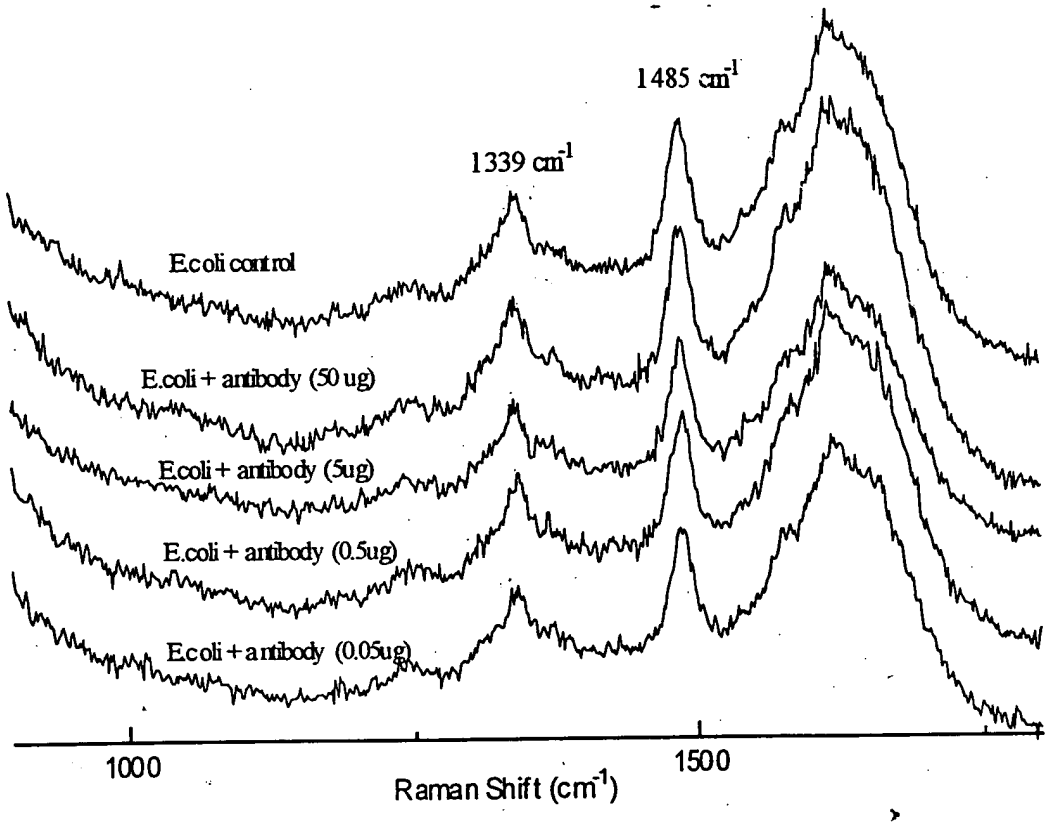

Figure 3. Raman Spectra of *E.coli*-Antibody Complexes with Different Amount of Excess Antibody